Investigations into Changes in Bone Turnover with Acute, Weight-Bearing Exercise in Healthy, Young Men

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ABSTRACT

Introduction/relevance to the Symposium: *Lower limb stress fracture injuries (SFx) account for a high number of working days lost during initial UK military training, cause considerable morbidity to recruits and contribute significantly to the high attrition from training.* ***Rationale:*** *Recent evidence from the assessment of circulating biochemical markers suggests that changes in bone turnover, a process in which old bone is removed (bone resorption) and new bone formed in its place might be involved in SFx development.* ***Methods and Results:*** *Blood-borne markers of bone resorption (C-terminal cross-linking telopeptide of type 1 collagen – β-CTX) and bone formation (N-terminal propeptides of procollagen type 1 – P1NP) and other bone-associated factors (parathyroid hormone – PTH, calcium, phosphate and osteoprotegerin – OPG) were measured before, during and up to four days after acute bouts of weight-bearing exercise. Investigations examined (i) the effect of training status (TS) on responses to exhaustive exercise; (ii) the effect of recovery duration (23 h vs 3 h; RD) between two bouts of moderate exercise; (iii) the effect of increasing exercise intensity (EI); and (iv) the effects of acute, pre-exercise feeding (PF). β-CTX, but not P1NP, was increased for four days following exhaustive exercise, but this response was not affected by TS. In contrast, two bouts of exercise separated by either 23 h or 3 h had no effect on β-CTX or P1NP. β-CTX but not P1NP was higher in the first hour post-exercise with exercise at the highest exercise intensity. PF suppressed resting β-CTX concentrations, although it did not suppress the rise in β-CTX with subsequent exercise but, compared with fasting, resulted in a greater increase. OPG was increased with exercise in all four investigations but this increase was not affected by TS, RD, EI or PF. Transient increases in PTH were seen with exercise in all studies. This increase was not affected by TS, RD, or PF but was increased at the highest exercise intensity only.* ***Conclusions:*** *Increased β-CTX but not P1NP following exhaustive exercise indicates an imbalance in the bone turnover process, favouring bone resorption, that lasts at least up to four days post-exercise and is unaffected by training status. In physically-active men, who have consumed an appropriate diet, two bouts of moderate exercise separated by either 23 h or 3 h has no effect on bone turnover markers. The effect of exercise on bone resorption, but not bone formation is, in part, dependent on exercise intensity, resulting in increased bone resorption with higher exercise intensity. Acute pre-feeding does not suppress the exercise-associated increase in bone resorption and might result in a less favourable change in bone turnover compared with fasting. Increases in OPG were not associated with changes in β-CTX, suggesting OPG is not an accurate reflection of changes in bone resorption with exercise. Increases in PTH and β-CTX suggest that the increase in PTH may play a role in the increased bone resorption seen with exercise. These data provide new information regarding changes in bone metabolism associated with weight-bearing exercise and may assist in modifying training practices to minimise unfavourable changes in bone turnover.*

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Introduction/relevance to the Symposium: Lower limb stress fracture injuries (SFx) account for a high number of working days lost during initial UK military training, cause considerable morbidity to recruits and contribute significantly to the high attrition from training. Rationale: Recent evidence from the assessment of circulating biochemical markers suggests that changes in bone turnover, a process in which old bone is removed (bone resorption) and new bone formed in its place might be involved in SFx development. Methods and Results: Blood-borne markers of bone resorption (C-terminal cross-linking telopeptide of type 1 collagen â-CTX) and bone formation (N-terminal propeptides of procollagen type 1 P1NP) and other bone-associated factors (parathyroid hormone PTH, calcium, phosphate and osteoprotegerin OPG) were measured before, during and up to four days after acute bouts of weight-bearing exercise. Investigations examined (i) the effect of training status (TS) on responses to exhaustive exercise; (ii) the effect of recovery duration (23 h vs 3 h; RD) between two bouts of moderate exercise; (iii) the effect of increasing exercise intensity (EI); and (iv) the effects of acute, pre-exercise feeding (PF). â-CTX, but not P1NP, was increased for four days following exhaustive exercise, but this response was not affected by TS. In contrast, two bouts of exercise separated by either 23 h or 3 h had no effect on â-CTX or P1NP. â-CTX but not P1NP was higher in the first hour post-exercise with exercise at the highest exercise intensity. PF suppressed resting â-CTX concentrations, although it did not suppress the rise in â-CTX with subsequent exercise but, compared with fasting, resulted in a greater increase. OPG was increased with exercise in all four investigations but this increase was not affected by TS, RD, EI or PF. Transient increases in PTH were seen with exercise in all studies. This increase was not affected by TS, RD, or PF but was increased at the highest exercise intensity only. Conclusions: Increased â-CTX but not P1NP following exhaustive exercise indicates an imbalance in the bone turnover process, favouring bone resorption, that lasts at least up to four days post-exercise and is unaffected by training status. In physically-active men, who have consumed an appropriate diet, two bouts of moderate exercise separated by either 23 h or 3 h has no effect on bone turnover markers. The effect of exercise on bone resorption, but not bone formation is, in part, dependent on exercise intensity, resulting in increased bone resorption with higher exercise intensity. Acute pre-feeding does not suppress the exercise-associated increase in bone resorption and might result in a less favourable change in bone turnover compared with fasting. Increases in OPG were not associated with changes in â-CTX, suggesting OPG is not an accurate reflection of changes in bone resorption with exercise. Increases in PTH and â-CTX suggest that the increase in PTH may play a role in the increased bone resorption seen with exercise. These data provide new information regarding changes in bone metabolism associated with weight-bearing exercise and may assist in modifying training practices to minimise unfavourable changes in bone turnover.

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1.0 INTRODUCTION

1.1 Military context

Stress fractures (SFx) are overuse injuries to bone that occur during repetitive loading. Small cracks develop in bones subjected to excessive loading and cause pain with exercise. Military recruits are susceptible to SFx of the lower limb, possibly due to the rapid onset of strenuous and often unaccustomed training. Lower limb SFx account for a high number of working days lost during initial training, cause considerable morbidity to recruits and contribute significantly to the high attrition from training. It is estimated that medical discharge of recruits due to SFx costs the MOD up to £2 million per annum for the Army alone (Personal communication, SO1, UK Army Training and Recruiting Agency).

The incidence of lower limb SFx reported during Phase-1 Army training approaches 10%, depending on the Army Training Regiment, and the risk of injury is higher in women than in men [1]. Reducing the number of lower limb SFx sustained during initial training would contribute significantly to the reduction in lost working days and medical discharges, but the precise mechanisms involved in the development of SFx are not fully understood. A clear understanding of the mechanism of SFx is required to enable the design and implementation of effective preventative strategies.

1.2 Theories of Stress Facture Development

Stress fractures arise from the inability of bone to withstand repetitive loading, and the development of micro damage leads to the mechanical failure of bone [2]. Two theories currently exist to explain how mechanical loading can lead to SFx. The first theory contends that SFx result from the development, accumulation and growth of microcracks, and are purely a result of mechanical damage [3]. This 'mechanical' theory of SFx development is not supported, however, by data from ex-vivo experiments of loaded bone specimens [4], and epidemiological data from populations with a high rate of SFx [5].

An alternative, now more widely accepted theory, holds that mechanical loading can directly result in the stimulation of targeted bone remodelling, a process in which old bone is removed (bone resorption) and new bone is formed in its place (bone formation), allowing the skeleton to continually adapt to the functional demands placed upon it. In this model, targeted remodelling occurs without the presence of microdamage and, as the process of bone resorption precedes that of bone formation, targeted bone remodelling results in transient, localised increases in bone porosity and a decrease in bone mass. A reduction in bone mass in these relatively 'osteoporotic' areas will, in turn, affect important mechanical properties such as stiffness, and continued loading will result in marked increases in stresses and strains, the accumulation of microdamage and eventually SFx [6].

1.3 Technological Advancements: New Insights into bone turnover

Changes in bone tissue associated with exercise may take months or even years to assess accurately using current scanning and imaging technologies. The recent availability of bone turnover markers (BTM) that reflect the bone resorption and formation processes, allow the investigation of acute changes in bone metabolism, and currently play an important role in the clinical understanding, monitoring and management of metabolic bone diseases. Markers of bone resorption, particularly C-terminal telopeptide region of collagen type 1 (β-CTX) increase rapidly after the menopause. Subsequently, in the newly increased number of resorption spaces, bone formation occurs that is reflected by an increase in serum levels of bone formation markers [7]. These bone formation markers, particularly N-terminal propeptides of procollagen type I (P1NP) also increase rapidly with the onset of intermittent treatment with bone formation-stimulating treatments [8], and show dose-dependent increases in line with increases in lumbar spine bone mineral density (BMD) with continued therapy [9]. Importantly, changes in these specific BTM, but not in previously used non-specific markers, predict fragility fractures independently of age, bone mineral density and prior fracture [10, 11]. Additionally, increased β-CTX – like a BMD T-score of <-2.5 or a previous fracture – is able to predict subsequent fracture in elderly populations [12]. Thus, using specific BTM, new insights into the acute effects of exercise on bone resorption and formation are possible.

1.4 Nutrition, energy availability and normal bone metabolism

While the importance of appropriate nutrition in maintaining normal bone turnover has long been recognised, it is only with the development of BTM and their monitoring during subsequent nutritional studies, that a more complete understanding of this relationship has been achieved. Almost all BTM as

well as many bone-related hormones, such as parathyroid hormone (PTH), display a circadian rhythm. This variation, particularly in β-CTX, is strongly linked to feeding patterns [13, 14], with the removal of nutrient intake substantially reducing the magnitude of the variation over a 24 h period. Subsequent studies of the acute ingestion of food have observed a rapid suppression of β-CTX concentrations for several hours thereafter and have implicated hormones released from the gut as mediating this process [15]. Other studies have shown an association between energy availability – the difference between energy intake and energy expenditure – and BTM concentrations. These studies show that when energy availability is reduced, bone formation marker concentrations are also reduced and, in the case of severe availability reductions, so are bone resorption markers, suggesting the interruption of normal bone turnover [16, 17]. Taken together, these data suggest that acute changes in nutritional status and their interaction with exercise are important factors in modulating the bone turnover process.

If changes in bone turnover, such as an increase in bone resorption or a decrease in bone formation, are involved in the development of SFx during training, interventions that favourably modify this imbalance might act as effective countermeasures. A better understanding of the effect of exercise, energy availability and recovery on specific bone turnover markers, under well-controlled conditions is, therefore, required.

2.0 MATERIALS AND METHODS

All studies were approved by the QinetiQ research ethics committee, and written informed consent was obtained for all participants. The studies were conducted according to the principles of the Helsinki Declaration [II]. Subjects were included if they were non-smokers, had not suffered a bone fracture of any type in the previous 12 months, were free from musculoskeletal injury, and were not taking any medication or suffering from any condition known to affect bone metabolism. Compliance with these inclusion criteria was confirmed from a medical screening questionnaire and a medical examination. All subjects report a history of some form of regular, weight-bearing activity.

2.1 General Methodology

All four studies used an identical general research design that included the standardisation of volunteers' activities from three days prior to until four days following the exercise interventions (Figure 2.1). During a first preliminary visit (P1), volunteers underwent a full medical examination to ensure they were suitable to participate in the studies. During a second preliminary visit (P2), volunteers had their maximal rate of oxygen uptake (VO_{2max}) measured to establish their physical fitness and allow the calculation of specific exercise intensities relative to VO_{2max} for use in the studies.

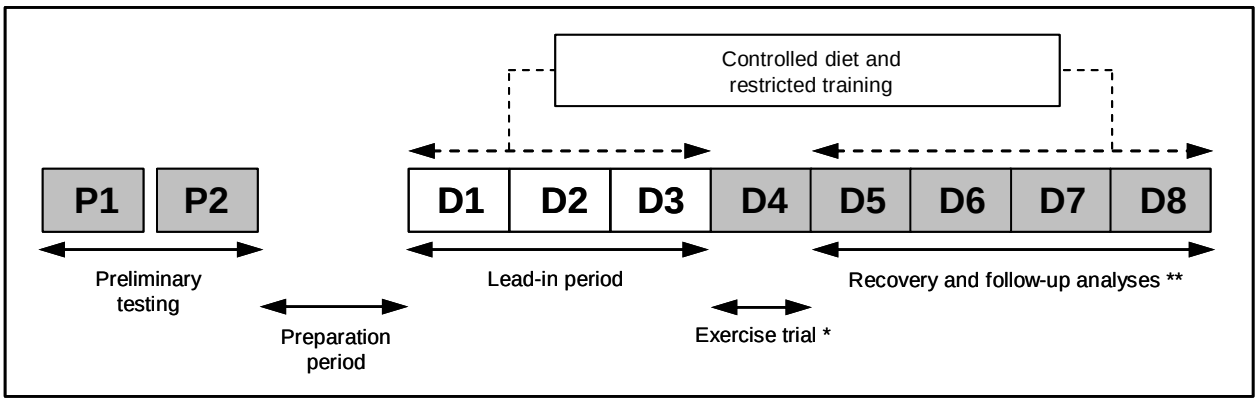

Figure 2.1 Outline of general study design. P – Preliminary days; D1 to D8 – Experimental days. Shaded boxes denote laboratory visits; adjoined boxes denote consecutive days. * D4 and D5 in Study II; ** D6 to D9 in Study II.

Volunteers then completed between one and three experimental trials over eight consecutive days (in Study II, this was nine days due to a 2 d exercise protocol on D4 and D5). For the first three days of this period, D1 to D3, volunteers refrained from all physical exercise and training. On the fourth day, D4 (D4 *and* D5 in Study II) subjects completed an exercise intervention on a motorised treadmill. On the four days following the exercise intervention, D5 to D8 (D6 and D9 in Study II), volunteers continued to refrain

from exercise and training and visited the laboratory each morning for follow-up analysis (+1 d to +4 d). A summary of the four studies is shown in Table 2.1.

Table 2.1 Overview of the experimental design of the four studies

Study Number and Title	Number of Conditions	Exercise Protocol	Comparison
STUDY I: Effect of training status (TS) on responses to acute, exhaustive exercise	1	Intermittent, exhaustive running at 65% to 70% VO_{2max}	Recreationally-active men (RA) *vs* endurance-trained male runners (ET)
STUDY II: Effect of recovery duration (RD) between two bouts of acute exercise	2	Two, 60 min bouts of running at 65% VO_{2max}	Recovery duration between exercise bouts: 23 h (LONG) *vs* 3 h (SHORT)
STUDY III: The Effect of Pre-feeding (PF) on responses to subsequent acute exercise	2	60 min of running at 65% VO_{2max}	An overnight fast (FAST) *vs* a standard breakfast (FED)
STUDY IV: The Effect of Exercise Intensity (EI) on responses to acute exercise	2	60 min of running	55% VO_{2max} (LOW) *vs* 75% VO_{2max} (HIGH)

2.2 Standardisation of Experimental Conditions

In order to control for the residual effects of prior exercise and the effect of any subsequent exercise, subjects were asked to refrain from any un-prescribed exercise from three days before the exercise intervention (D1 to D3) until all analysis was complete on the fourth follow-up day. In order to control for changes in markers of bone metabolism related to their circadian rhythms, baseline blood samples and those taken on the four follow-up days were all collected early in the morning. To ensure normal nutritional status during each experimental trial, volunteers consumed a controlled diet. This diet was designed on an individual basis for each volunteer, based on their self reported dietary habits. In addition, to control for the acute effects of feeding, especially on β-CTX, baseline blood samples and those taken on the four follow-up days were collected after subjects had fasted from 9:00 p.m. the previous evening. All blood samples were analysed for β-CTX, P1NP, PTH, albumin-adjusted calcium (ACa), phosphate and osteoprotegerin (OPG).

2.7 Sample collection

For measurement of β-CTX, P1NP and OPG, blood was transferred into pre-cooled tubes containing 15%, 0.12 ml of K_3E EDTA (Becton Dickinson Vacutainer System, USA). Tubes were inverted 8 to 10 times and centrifuged immediately. For measurement of PTH, ACa and phosphate, blood was transferred into precooled standard tubes (Becton Dickinson Vacutainer System, USA) and left to clot at room temperature for 60 min before centrifuging. All tubes were centrifuged at 2000 rpm and 5°C for 10 min, samples separated and aliquots stored at -70°C until analysis.

2.8 Analytical Methods

β-CTX was measured using an electrochemiluminescent immunoassay (ECLIA) on an Elecsys 2010 immunoanalyser (Roche, Lewes, UK). Inter-assay coefficient of variation (CV) was <8% between 0.2 and 1.5 $ng \cdot mL^{-1}$. The assay sensitivity (replicates of the zero standard) was <0.01 $ng \cdot mL^{-1}$. P1NP was measured by radioimmunoassay (RIA) supplied by Orion Diagnostica (Espoo, Finland). This assay has a sensitivity of 4 $\mu g \cdot L^{-1}$ established from precision profiles (22% CV of duplicates) and an inter-assay CV of 3.5 - 5.4% across the concentration range 10 - 250 $ug \cdot L^{-1}$. OPG was measured using a commercial solid phase enzyme linked immunosorbent assay (ELISA) (Supplied by IDS Boldon Tyne & Wear UK). The assay has a detection limit of 0.14 $pmol \cdot L^{-1}$ and an inter / intra assay CV of <10% across the range 1 - 30 $pmol \cdot L^{-1}$. Intact parathyroid hormone was measured using a commercial immunometric assay (Nichols Institute, San Juan, Capistrano, CA) with a detection limit of 0.5 $pmol \cdot L^{-1}$ and inter-assay and intra-assay CV of <5% across the range 1 - 40 $pmol \cdot L^{-1}$. Calcium was measured using a standard commercial assay supplied by Roche (Lewes, UK) performed on a Roche Modular Analytical System. The range of measurement in serum/plasma is 0.05 - 5.00 $mmol \cdot L^{-1}$. Albumin was measured using a standard

commercial assay supplied by Roche (Lewes, UK) performed on a Roche Modular Analytical System. The range of measurement in serum is 10 - 70 $g \cdot L^{-1}$. Phosphate was measured using a standard commercial assay supplied by Roche (Lewes, UK) performed on a Roche Modular Analytical System. The range of measurement in serum/plasma is 0.10 - 6.46 $mmol \cdot L^{-1}$.

2.9 Statistical Analysis

All data are presented as mean ± 1SD unless otherwise stated. Statistical significance was accepted at an alpha level of $P<0.05$. All biochemical data were analysed using a linear mixed model analysis of variance (LMM), with the factors *Time* and *Group* included and with individuals as a random within-group factor. The assumptions of the ANOVA were investigated by examining the distribution of residuals and the pattern of residuals versus fitted values. Where non-normality or non-constant variance was observed, a transformation was applied to the data so that the assumptions were satisfied.

Where there was a significant main effect of *Time* but no significant *Group* x *Time* interaction, each subsequent time point was compared against BASE using a pooled mean from all groups using Student's t-tests for paired data with a Holm-Bonferroni correction. When the *Condition x Time* interaction was significant, within each group each subsequent time point was compared against BASE as described above and individual groups were compared to each other at all time points using Student's t-tests for paired or unpaired data as appropriate with a Holm-Bonferroni correction.

3.0 RESULTS

3.1 Bone turnover markers

Study I: β-CTX concentrations increased significantly from baseline on each of the four follow-up days in both the recreationally-active (RA) and endurance-trained (ET) groups (Figure 3.1, Panel A). Peak β-CTX occurred at +1 d in RA (43 ± 19 %) and +2 d in ET (46 ± 31%) but there was no effect of training status (TS) on the β-CTX response. There was no significant change in P1NP concentrations in the RA, ET groups and no effect of TS (Figure 3.1, Panel B).

Study II: There was no change from baseline in β-CTX concentrations in either the LONG or SHORT condition and no effect of recovery duration (RD) (Figure 3.1, Panel C). P1NP concentrations were also unchanged in response to either protocol and there was no effect of RD (Figure 3.1, Panel D).

Study III: Prior to exercise, pre-feeding (PF) resulted in a greater ($P<0.001$) reduction in β-CTX (45% *vs* 30%) compared with fasting (Figure 3.1, Panel E). From pre-exercise to 1 h post-exercise, β-CTX increased 86 ± 40 % in FED compared with 22 ± 18% in FAST so that concentrations were similar to baseline in both groups. β-CTX concentrations were higher ($P<0.05$) than BASE in FED at +1 d but were not different from FAST from +1 d to +4 d. Prior to exercise, P1NP was unchanged in FAST but showed a small, but significant ($P<0.01$) reduction in FED resulting in lower ($P<0.01$) P1NP in FED compared with FAST (Figure 3.1, Panel F). By 1 h post-exercise, P1NP concentrations were not different from BASE in either group and there were no changes in P1NP from +1 d to +4 d.

Study IV: β-CTX concentrations declined in LOW during exercise and up to 1 h of recovery (Figure 3.1, Panel G). In contrast, there was no decrease in HIGH in this period, resulting in higher β-CTX concentrations in HIGH compared with LOW in the first hour post-exercise. β-CTX was not different from baseline in either group from +1d to +4 d. P1NP concentrations were increased ($P<0.001$) at EX60 compared with BASE, but not thereafter (Figure 3.1, Panel H). The greatest increase was observed in HIGH but there was no effect of exercise intensity on P1NP concentrations.

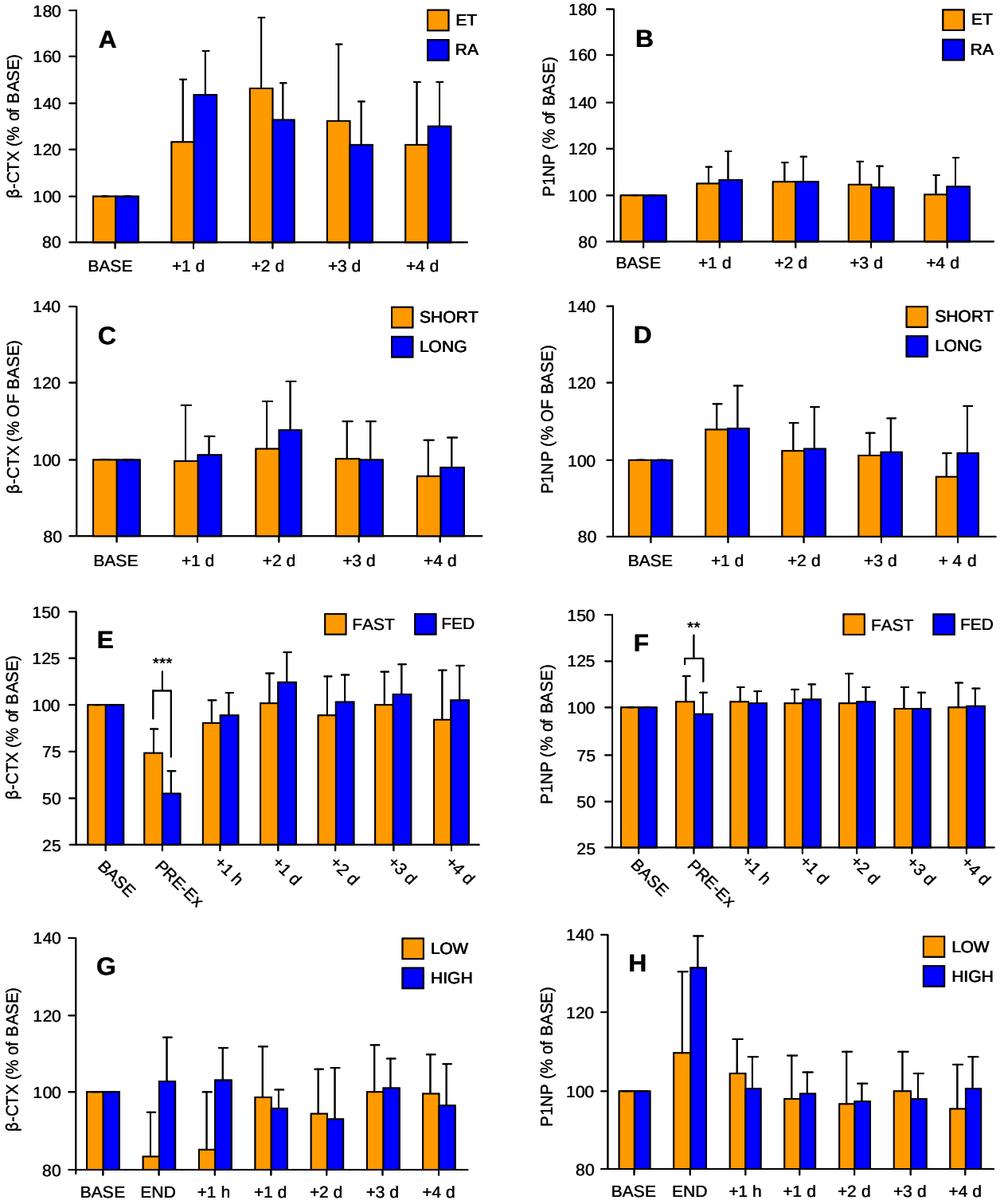

Figure 3.1. Percentage change from baseline (BASE) in β-CTX and P1NP in Study I (Panel A and B), Study II (Panel C and D), Study III (Panel E and F) and Study IV (Panel G and H). RA – recreationally-active; ET – endurance-trained; LONG – 23 h recovery; SHORT – 3 h recovery; FAST – overnight fast; FED – standardised breakfast; LOW – 55% VO_{2max}; HIGH – 75% VO_{2max}. Values are mean ± 1SD. ** different (P<0.01); *** different (P<0.001).

3.2 Osteoprotegerin (OPG)

Study I: OPG concentrations increased (P<0.05) from baseline during exercise and remained elevated (P<0.01) at 2 h post-exercise (Figure 3.2, Panel A). Concentrations were no longer different from baseline at +1 d and there was no effect of TS.

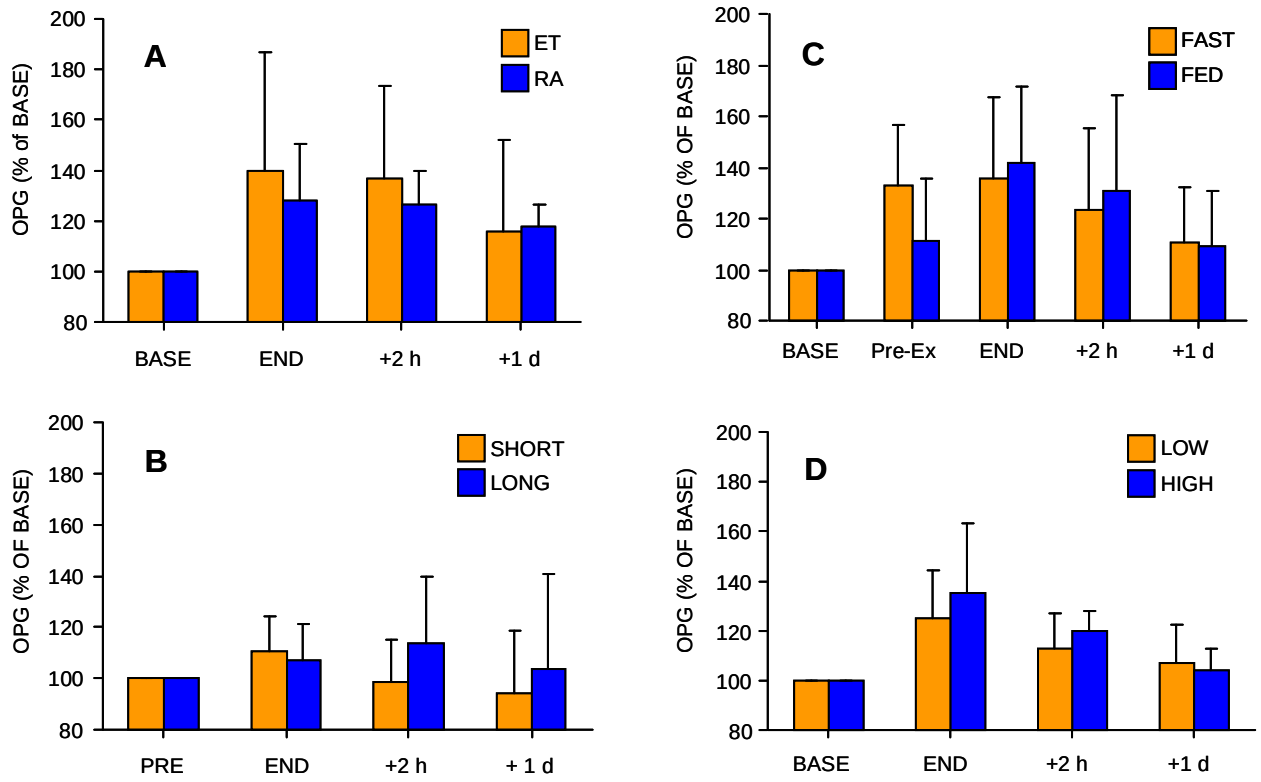

Figure 3.2. Percentage change from baseline (BASE) in OPG in Study I (Panel A), Study II (Panel B), Study III (Panel C) and Study IV (Panel D). RA – recreationally-active; ET – endurance-trained; LONG – 23 h recovery; SHORT – 3 h recovery; FAST – overnight fast; FED – standardised breakfast; LOW – 55% VO_{2max}; HIGH – 75% VO_{2max}. Values are mean ± 1SD.

Study II: OPG concentrations increased (P<0.05) during both protocols, but the response to a second bout of exercise was not affected by RD (Figure 3.2, Panel B).

Study III: Prior to exercise, OPG concentrations were not effected by feeding (Figure 3.2, Panel C). OPG increased with exercise and remained elevated at 2 h post-exercise but this increase was not affected by PF.

Study IV: OPG concentrations were increased (P<0.05) during exercise in both groups and remained elevated (P<0.05) at 2 h post-exercise but there was no effect of EI (Figure 3.2, Panel D).

3.3 Markers of calcium metabolism

Study I: PTH was increased (P<0.001) at the end of exercise in RA and ET but this increase was not affected by TS (Figure 3.3, Panel A). Concentrations decreased rapidly following exercise and were not different from baseline at 30 min post-exercise in either group. ACa increased (P<0.001) with exercise in both groups although the increase was greater in ET than RA, resulting in higher (P<0.05) post-exercise ACa concentrations. Phosphate was increased (P<0.05) by exercise in both groups but this increase was not effected by TS.

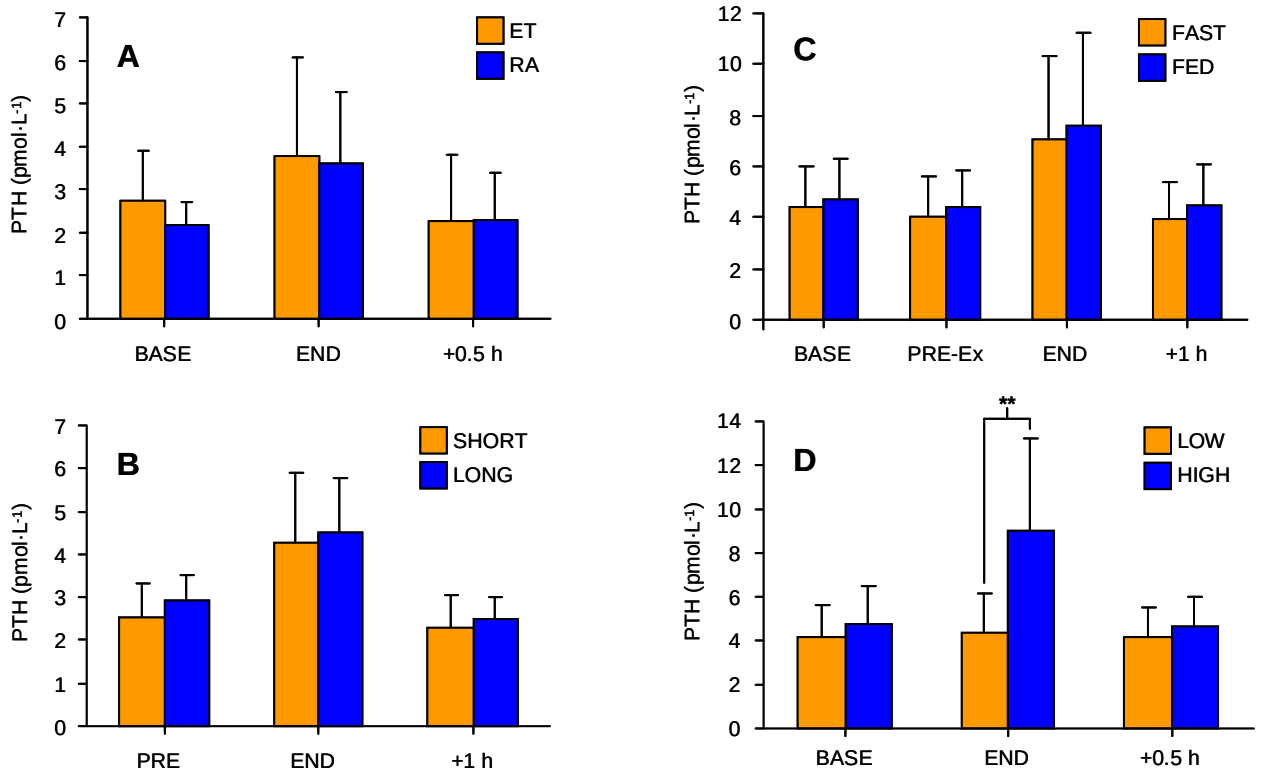

Figure 3.3. Changes in PTH in Study I (Panel A), Study II (Panel B), Study III (Panel C) and Study IV (Panel D). RA – recreationally-active; ET – endurance-trained; LONG – 23 h recovery; SHORT – 3 h recovery; FAST – overnight fast; FED – standardised breakfast; LOW – 55% VO_{2max}; HIGH – 75% VO_{2max}. Values are mean ± 1SD. ** different (P<0.01).

Study II: PTH was increased (P<0.05) with all exercise bouts, although the increase with a second bout of exercise was not affected by RD (Figure 3.3, Panel B). PTH concentrations were not different from BASE at 1 h post-exercise. ACa increased (P<0.001) with exercise, although the increase with a second bout of exercise was not affected by RD. Due to a large number of haemolysed samples, insufficient data was available for analysis of phosphate responses to the two exercise protocols.

Study III: PTH decreased (P<0.01) prior to exercise in both groups prior to exercise but this decrease was not affected by PF (Figure 3.3, Panel C). PTH increased with subsequent exercise in both groups but this increase was not affected by PF. Concentrations were not different from BASE at 1 h post-exercise. There was no significant change in ACa in either group in response to feeding or exercise. Phosphate decreased (P<0.05) prior to exercise, and increased (P<0.05) during exercise, but neither variation was affected by pre-feeding.

Study IV: There was no change in PTH in LOW (Figure 3.3, Panel D). In HIGH, PTH concentrations increased with exercise and were higher (P<0.01) in HIGH than low at the end of exercise. Concentrations were not different from BASE at 30 min post-exercise. ACa was increased (P<0.05) during exercise in both conditions but this increase was not affected by EI. Phosphate concentrations were increased (P<0.05) by exercise but this increase was not affected by EI.

4.0 DISCUSSION

This is the first series of laboratory-based investigations that has examined changes in the same specific bone turnover markers (BTM) as well as other markers of bone metabolism under tightly controlled conditions and over an identical timescale.

4.1 The effects of acute exercise on bone turnover markers

In Study I, increased β-CTX concentrations, but not P1NP concentrations, for four days following acute exercise suggests that, in fasted, recreationally-active men, acute, exhaustive, weight-bearing exercise results in an imbalance in the bone turnover process and a negative remodelling balance. This imbalance will likely result in a transient weakening of bone, which might increase the risk of micro-damage development if further weight-bearing activities are performed during this period. The similar pattern of change in bone turnover markers (BTM) in recreationally-active and endurance-trained men, when exhaustive exercise was performed at the same relative workload (65 to 70% of VO_{2max}), suggests that the magnitude and duration of the imbalance is not altered by improved training status. If, however, exercise is set in absolute terms (*e.g.* running at a 10 min·mile^{-1} pace), relative intensity would decrease with increased physical fitness. Given the results of Study IV, where β-CTX concentrations were higher, albeit transiently, with exercise at 75% compared with 65% VO_{2max}, it is possible that improved training status, and the associated reduction in relative exercise intensity, would result in a beneficial effect on alterations to bone turnover.

The results of Study II showed that repeated bouts of moderate exercise separated by either 23 h or 3 h, have no effect on bone turnover markers when measured over the same period as that in Study I. These findings suggest that neither protocol results in a negative alteration in bone turnover, and that a balance between bone resorption and bone formation is maintained, even when the recovery period between two bouts of exercise is reduced to only 3 h. Several factors should, however, be considered, specifically that in this study 1) subjects were all in good physical condition with a history of regular, weight-bearing exercise; 2) subjects performed no exercise for three days prior to, and for four days after either protocol, and; 3) subjects consumed a diet isocaloric with their normal diet, that was adjusted for the increased energy expenditure due to exercise. These results, therefore, may not be applicable to persons of below average physical condition with little or no history of weight-bearing exercise (*e.g.* some recruits during the early stages of training), while the effect of exercise completed using either schedule on BTM during a longer period of regular repeated exercise, is unknown. In addition, nutritional intake by recruits during UK Army basic training has been shown to be inappropriately low for the demands of training [18], while energy restriction during repeated exercise has been shown to result in a negative remodelling balance [19]. It is also possible, therefore, that changes in BTM in energy restricted persons undertaking exercise using either of the two schedules may be different than those reported here.

Acute feeding has been shown to suppress resting concentrations of β-CTX [15, 20]. Study III was, therefore, designed to establish whether this effect could be use to favourably modulate changes in bone turnover markers with subsequent exercise. In doing so, this might also in part explain the different β-CTX responses to Study I and Study II as in Study I subjects were fasted prior to exercise, whereas in Study II exercise was always performed following one or more standardised meals. As anticipated, acute feeding suppressed resting β-CTX concentrations but did not suppress the rise in β-CTX seen with subsequent acute exercise. When assessed over the same time period as in Studies I and II (1 to 4 days post-exercise), however, there was no effect of either protocol on bone turnover markers, suggesting that the differing nutritional states of subjects prior to exercise in these studies is unlikely, on its own, to explain the different β-CTX responses.

The results of Study III suggest that, although the absolute increase in β-CTX from pre-exercise is greater with feeding, feeding or fasting prior to exercise result in similar post-exercise concentrations of BTM suggesting that PF is not effective in suppressing the exercise-associated in bone resorption. Although recruits might, on occasion, miss a meal due to time constraints, it seems unlikely that physical training sessions would be deliberated scheduled directly following an extended fast (*e.g.* early in the morning, prior to breakfast). The response to acute exercise in the FED condition state would, therefore, likely be a more typical response to exercise during military training. Thus, future studies of nutritional interventions might investigate alternative strategies (*e.g.* continuous nutrition or nutrition targeted at the late

exercise/early recovery period) and consider examining them in a pre-fed state that might better reflect the training environment.

Results of Study IV suggest that the effect of exercise on bone resorption is, in part, dependent on exercise intensity. This effect might, in part, explain the differences between changes in bone resorption between Studies I and II, as exercise intensity was greater in the later part of Study I. The transient effect of exercise intensity and the lack of any effect of increasing exercise intensity on bone resorption when measured from 1 to 4 d post-exercise, suggests that exercise intensity alone is unlikely to fully explain the different β-CTX responses in Studies I and II. Study IV shows an increase in P1NP immediately after exercise, particularly with higher intensity exercise. It would seem unlikely, however, that this increase represents a direct effect of exercise on bone formation but might instead reflect accelerated efflux of existing P1NP into the circulation from tissues containing type 1 collagen including the muscle, tendon and bone. Like Studies I to III, the lack of any change in P1NP on the recovery days in Study IV suggests no effect of exercise on bone formation up to 4 d after exercise.

An effect of exercise intensity on changes in bone resorption with acute exercise might have important implications for military training. Previous data from Phase 1 training reports considerable variation in relative exercise intensity both within sexes and between the sexes during 'common' activities, with those with lower physical fitness levels attaining higher relative exercise intensities [21]. Based on our results, people who more frequently experience higher exercise intensities might more frequently experience unfavourable changes in bone turnover during periods of repeated bouts of acute exercise. This might, in part, explain why physical fitness on entry into training is a risk factor for SFx [1]. Rayson et al. [21] shows female recruits tending to experience higher relative exercise intensities compared with their male counterparts. With the introduction of single-sex training in the UK Army in 2006, average relative exercise intensities for female recruits have reduced, although for men, the total physical demand of training is greater in all male platoons than in mixed platoons [22]. This is possibly due to overall higher exercise intensities during physical activities where the more aerobically fit males are not 'held back' by their less fit female counterparts [21]. It is possible, therefore, that single-sex training might actually increase the variation in relative exercise intensity between the least and most aerobically fit male recruits and possibly increasing the number of male recruits suffering unfavourable alterations in bone turnover during exercise. No data is, however, currently available on the effect of single-sex training on SFx incidence in men.

4.2 The effect of acute exercise on systemic OPG and its role in changes in bone turnover

In bone, osteoclast development is stimulated by RANKL/RANK binding with OPG acting as a decoy receptor, binding to RANK and down-regulating RANKL/RANK signalling [23-25]. OPG is detectable in serum and increases in the circulating concentration in conditions associated with significant bone loss have been interpreted as a compensatory response to increased bone resorption [26, 27].

This series of studies are the first to examine the time-course of changes in serum OPG with exercise as well as measure OPG concomitantly with BTM. Although these studies demonstrate increases in serum OPG concurrently with changes in β-CTX, differences in the time-courses of changes in OPG and β-CTX were, however, evident in Study I, while in Study II OPG was increased while β-CTX was not. In addition, reduced β-CTX with feeding in Study III and higher β-CTX with higher exercise intensity in Study IV were not accompanied by similar changes in OPG.

The temporal association of changes in β-CTX and OPG with exercise is not known, and there is little data on the temporal association between changes in β-CTX – or indeed other bone turnover markers – and OPG along time scales similar those studied here [28]. When taken together, however, these findings suggest that changes in serum OPG might not reflect changes in bone resorption but might be a direct effect of exercise. Importantly, it remains to be clearly established to what degree circulating OPG accurately reflects local OPG production in bone, as OPG is also expressed in the kidney, lung and heart [29] and, in addition to correlating with markers of bone turnover [29, 30], serum OPG is also associated with both markers of diabetes and cardiovascular mortality [31].

4.3 The effect of acute exercise on calcium metabolism and its role in changes in bone turnover

These studies have confirmed that acute, weight-bearing, endurance exercise is associated with a marked, but transient increase in PTH concentrations. The magnitude of this increase is not influenced by training status, recovery duration from prior exercise, or acute pre-exercise nutrient ingestion, but may, in

part, be determined by exercise intensity. PTH serves to maintain the calcium concentration of extra-cellular fluids through its actions at the kidney and the skeleton, where it is the main hormone regulating bone remodelling. Elevated PTH is associated with increased bone resorption and changes in both β-CTX and PTH in Study I and Study III, and particularly in Study IV, suggest that the increase in PTH may play a role in the increased bone resorption seen with exercise.

When given exogenously, however, PTH has been shown to have dual effects on bone, with prolonged infusions resulting in increased bone resorption and bone loss, while daily injections – which produce transient spikes in PTH concentrations – induce bone formation [32]. The rapid resolution of PTH concentrations is an important feature of its anabolic effect on bone during intermittent administration [33] and results from this work programme suggest that if acute exercise was repeated daily (*e.g.* during training), a profile of intermittent PTH increases would exist. Although this would suggest that the transient increases in PTH with exercise might produce anabolic effects on bone, exercise tended to be associated with increased bone resorption rather than bone formation.

Of the other factors known to increase PTH concentrations at rest, a reduction in serum calcium does not appear to be involved as ACa was either unchanged or increased with exercise in all four studies. Increased phosphate is also known to increase resting PTH concentrations [34]. Increases in phosphate were seen with exercise in all studies, although in Study IV, increased PTH with high intensity exercise only, despite similar increases in phosphate at all intensities. This suggests that factors other than phosphate determine the PTH response to exercise.

4.4 Appropriate scheduling of exercise activities based on changes in bone turnover markers with acute exercise

The clinical significance of the increase in β-CTX but not P1NP during the four days following exhaustive exercise in Study I is not clear, but suggests that during this period, bone could be considered to be at 'increased risk' of damage from further mechanical loading. There is currently, however, insufficient data to offer precise guidelines on appropriate training schedules, since the specific factors (*e.g.* duration and intensity of exercise, energy substrate depletion) that result in alterations in bone turnover under these conditions remain to be identified. Although results from Study II suggest that two bouts of moderate exercise conducted on the same day or on consecutive days has no negative effect on bone turnover markers, the effect of an additional bout of exercise in the period following a highly strenuous bout that results in an increase in bone resorption (*e.g.* Study I) remains unknown. Further activities that contain high mechanical loads (*e.g.* drill) or both metabolic 'stress' and mechanical loading (*e.g.* running, marching) may not be appropriate during this period, although the potential increase in SFx risk with such activities cannot yet be quantified.

In conclusion, increased β-CTX but not P1NP following exhaustive exercise indicates a negative remodelling balance, which lasts at least up to four days post-exercise. This imbalance is unaffected by training status when exercise is prescribed at the same relative workload. In physically-active men, who have consumed an appropriate diet, two bouts of moderate exercise separated by either 23 h or 3 h have no effect on bone turnover markers. Although acute feeding suppresses resting β-CTX concentrations, pre-feeding does not suppress the increase in β-CTX associated with subsequent, acute exercise. The effect of exercise on bone resorption, but not bone formation is, in part, dependent on exercise intensity, resulting in transiently higher β-CTX in the first hour post-exercise with higher exercise intensity. Increases in serum OPG were not consistently associated with changes in β-CTX, suggesting that OPG might not be an accurate reflection of changes in bone resorption with exercise. Increases in PTH may be responsible for increases in bone resorption but, unlike at rest, this increase cannot be explained by decreased calcium or increased phosphate. These data provide new information regarding changes in bone turnover associated with acute, weight-bearing exercise and may assist in modifying military training practices to minimise unfavourable changes in bone turnover.

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